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Output O.T3.1
Ex situ gene stocks of Danube sturgeons

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1. Introduction

Sturgeon populations represent an important historic, economic and natural heritage of the Danube River Basin and are prone to serve as indicators of good ecological status. Their products such as meat and caviar are well known rendering these species part of tradition and culture of the Danube Region. Sturgeons are also sensitive animals, which indicate environmental pressures. As flagship-species, they are valuable indicators for the health of river ecosystems. However, their populations suffered substantial losses from decades of overfishing, habitat fragmentation and pollution (Guti 2006) mainly in the late 20th and early 21st centuries. Fragmentation of the migratory route creates an ecological barrier restricting the habitat to be used and isolating population segments while permitting only one-way gene flow. The strongest effects upon longitudinal connectivity are resulting from the Iron Gate Dams and the Gabčikovo Dam but multiple smaller dams fragment the Upper Danube and the tributaries throughout the catchment (Friedrich et al. 2019). Furthermore, habitat degradation is a potent threat, as side arms and sections with slower current velocities historically provided feeding and nursing grounds for migratory species, but drainage and river control measures largely eliminated these sections. Furthermore, new threats of invasive species and global climate change are likely to exert even more pressures on these species and their protection is more urgently required than ever before.

The goals of habitat protection, restoration of migratory routes, restocking events and different educational and strategic approaches have been outlined by the ICPDR and its Sturgeon 2020 program. This program was followed by the network of national and international stakeholders in the "Danube Sturgeon Task Force". The DSTF is linked to the ICPDR and the Priority Area 6 of the EU Strategy for the Danube Region (Biodiversity and Landscape Diversity), which harmonizes sectoral policies between environmental protection and economic requirements.

The DTP2-038-2.3 – MEASURES project is part of the Interreg Danube Transnational Programme and was designed to promote the functionality of ecological corridors in the Danube River Basin. The stability of migratory fish populations requires long-term restoration of river connectivity however the populations decrease at such speed, that immediate short-term protection is necessary to ensure the survival of the species in question. The MEASURES project targets both time-scales and reviews the knowledge and practical solutions that serve these issues. Although multiple species of migratory fish are accounted for in the MEASURES project, sturgeons are especially targeted by additional measures.

One of the most straightforward actions for protecting endangered species, is to create *ex situ* stocks of representative entities of populations in question in a safe and controlled environment to maintain ecologically viable population sizes in such facilities. Thus, providing a backup for the dwindling natural populations under controlled conditions. These serve as a basis for their reintroduction once the conditions if the natural habitats are sufficiently suitable. This method has significant value for protecting individuals, but also provides the basis for controlled propagation and restocking. During these occasions large quantities of genetically controlled, viable individuals are released into the natural habitats to support or establish wild populations. These actions not only strengthen natural populations, but provide a visible platform to engage with stakeholders and public audience about the issues of migratory fish species and eco-corridors.

Of the six sturgeon species, native to the Danube River Basin (*Huso huso, Acipenser gueldenstaedtii, A. nudiventris, A. ruthenus, A. stellatus, A. sturio*), the sterlet (*A. ruthenus*) and the Russian sturgeon (*Acipenser gueldenstaedti*) were selected in the frame of the MEASURES project to strengthen their natural populations through pilot restocking and genetic analysis of wild specimens and captive broodstock.

2. Establishing *ex situ st*ocks of Danube sturgeons

Due to the endangered nature of the Danube sturgeon species, sufficiently sized and diverse *ex situ* stocks, representing the full width of plasticity of the wild populations, maintained under controlled conditions are key for their survival. These *ex situ* stocks provide the basis for annual artificial reproductions, comparison of stocks by genetic profiling and potential for releasing vulnerable fish species in their natural environment. For the maintenance of *ex situ* stocks, it is vital to collect wild specimens. These individuals can contribute genetically to the diversity of the already existing *ex situ* populations when they recruit into the broodstock. It is also important that for restocking purposes, wild fish in the *ex situ* stock must originate from the same region where the planned release is to take place (genetic integrity). Collecting wild specimens is followed by biometric and genetic analysis as well. These actions help to ensure a proper phenotypic and genetic background for the selected breeders in the gene stock, and consequently, long-term genetic stability of both *ex situ* population as well as the released individuals.

3. Sterlet *ex situ* stock

NAIK HAKI has a long history in genetic *ex situ* conservation management. An adequate population size of the sterlet is available for reproduction and releases or for supplementary expansion of the *ex situ* stock.

The *ex situ* sturgeon broodstock represents great value due to the long-term efforts that have been required to establish it. As such its maintenance requires above average safety (provision more careful rearing conditions than in aquaculture production; low stocking, proper feeding, optimal water quality variables, etc.) and tracking measures. For tracking purposes, each individual has its own PIT tag to identify and trace the *ex situ* stock at the individual level. The genetically analyzed sterlet broodstock (not the whole *ex situ* stock) in this study originates from the municipal district of Leányfalu, Ercsi, Baja, Mindszent and Kisköre. The first three of these locations can be found at the Hungarian sections of the Middle-Danube, the last two are from the Tisza River, which is the largest tributary of Danube in Hungary.

3.1 Broodstock reinforcement and gene bank maintenance

In project MEASURES, NAIK-HAKI attempted to capture sterlet broodstock from the Middle-Danube section in 2019 and 2020, but only when additional help of external fishermen was utilized in 2020, the attempt was successful. The institute managed to capture additional 10 sterlets (originate from Leányfalu, Danube) prior the spawning season and transfer them to NAIK-HAKI into controlled conditions, where they successfully survived and adapted to the different environment in the upcoming critical months. The sterlets had 1.73 kg average weight per individual, which is a size generally smaller than the generic broodstock population, and only 2 of them were sufficiently mature to be used in the propagation procedure in 2020. This catch was used to expand the existing sterlet *ex situ* stock at NAIK-HAKI. Including the new 10 Danubian individuals, NAIK-HAKI genotyped 144 sterlet brooders (3,03 kg average weight per individual) in 2020.

Reproduction planning and theoretical considerations

In the MEASURES project, there were two artificial propagation events at NAIK-HAKI which were planned to increase the sterlet stocks available. For reproduction purposes, in an ideal case we choose 15 “ready to spawn” (which were not reproduced in the previous year) specimens from each sex to ensure the appropriate genetic diversity in the next generation.

Reproduction preparations

From those 30 selected individuals at least 20 (10 females and 10 males) are designated for propagation to contribute to the next generation. The selected broodstock are transferred from their maintenance pond to hatchery tanks 4-5 m^3 each with the same temperature, 6-8 mg/L dissolved oxygen concentration and pH 7.5-8. If the external water temperature is lower than 14°C then slow raising in water temperature (max 0.5°C/day) has to be applied.

Timing and induction of reproduction

For induced reproduction we use analogues of mammalian luteinizing hormone-releasing hormone, which is the des-Gly10(D-Ala6)-LH-RH Ethylamid. This hormone has been successfully used in several sturgeon species and the dose at 40 μg/kg for females can be used to induce final oocyte maturation in sterlet.
On 14°C 34-46 hours after a successful induction eggs are collected by an incision of the caudal section of the oviducts. Milt is obtained from the males by pressing the abdominal wall. The stickiness of the fertilized eggs is eliminated by a talc-water solution (500g talc/10L water). Eggs are incubated in Zug jars, 300-400 g fertilized, treated (not sticky) eggs can be loaded in a Zug jar with the volume of 7L. The water flow rate during incubation is around 1.5-2.5 L/min. Polyvinylpyrrolidone (PVP) Iodine treatment was used to control fungus in

every 12 hours during incubation with 10 mL in each Zug jar for 10 minutes without water flow. The hatching starts about five days after fertilization at 16-16.5°C.

During hatching larvae are transferred to larger tanks (preferably circle tank) with shallow water depth (30-50 cm) and lower water flow (5L/min). The onset of exogenous feeding takes place around 9 dph (day of post hatch) when the melanin plug being evacuated from the gastrointestinal tract.

In the first days of active feeding larvae are fed with *Artemia nauplii* or fine-chopped Chironomid worms.

The nursing largely depends on the method and efficiency of weaning to artificial diets. The weaning of sterlet batch for release starts around 25 dph. The duration of weaning is usually 3-4 days, when the quantity of natural food is gradually decreased and that of artificial feed is increased. Some of these feeds have been adapted from salmonid culture however, special starter feeds for sturgeons are also manufactured. The protein content of the applied feeds is between 45-50% with particle size of 0.2-0.8 mm. For releasing purposes the adequate portion of natural food is further provided at least once a day, parallel to the artificial diet.

3.2. Sterlet reproduction in 2019

In total 27 sexually matured individuals were selected and transported to the hatchery. From the selected individuals 16 were females and 9 were males. After the hormone induction, eggs from 9 ovulated females were successfully stripped and milt from 5 males were collected (**Table 1**). The motility of the milt was checked and only the motile sperm was mixed prior to fertilization. In total 7,790 g of dry eggs were obtained. After successful fertilization the average fertilization rate was 49.2%.

Table 1. General description and notes for sterlet reproduction in 2019

Sex	Species	PIT tag ID	01/04/2019	09/04/2019	Notes
			Weight (kg)	Reproduction	
♀	Sterlet broodstock	0415D5026D	5,10	✓	1060 g eggs
	Sterlet broodstock	0415D527CA	3,40	×	no eggs
	Sterlet broodstock	452F5B5148	2,90	×	no eggs
	Sterlet broodstock	0415D4C256	2,95	✓	529 g eggs
	Sterlet broodstock	0415D4F4F1/4AA64	4,75	×	no eggs
	Sterlet broodstock	0415D51BD4	4,60	×	no eggs
	Sterlet broodstock	0415D521F8	4,70	×	no eggs
	Sterlet broodstock	0415D50028	2,70	×	No eggs
	Sterlet broodstock	4349763139	5,00	✓	810 g eggs
	Sterlet broodstock	0415D52286	2,10	✓	460 g eggs
	Sterlet broodstock	0415D5270D	4,00	✓	948 g eggs
	Sterlet broodstock	0415D4FF49	3,60	✓	990 g eggs/died
	Sterlet broodstock	0415D51ABF	4,10	✓	1400 g eggs
	Sterlet broodstock	0415D51143	2,40	✓	663 g eggs
	Sterlet broodstock	0415D52C4C	3,80	✓	930 g eggs
♀♂	Sterlet broodstock	4461467344	2,50	×	hermaphrodite
♂	Sterlet broodstock	0415D5074D	1,80	✓	
	Sterlet broodstock	0415D508F7	4,50	×	no sperm
	Sterlet broodstock	0415D50826	4,40	✓	
	Sterlet broodstock	0415D4A984	2,40	×	no sperm
	Sterlet broodstock	434857336A	3,50	×	no sperm
	Sterlet broodstock	0415D529B6	4,00	✓	
	Sterlet broodstock	0415D52CC5	3,25	×	no sperm
	Sterlet broodstock	0415D4A0CB	3,50	×	no sperm
	Sterlet broodstock	0415D5208F/525EF	1,90	✓	
	Sterlet broodstock	4534647709	1,80	×	no sperm
	Sterlet broodstock	0415D500ED	1,30	✓	

3.3. Sterlet reproduction in 2020

In 2020, only 2 of the captured 10 sterlets from Leányfalu proved to be matured enough for reproduction. For reproduction we selected 15 “ready to spawn” females from the gene bank and 2 Danubian wild females, in case of males we selected 12 from the gene bank and got 8 from the Danube. We successfully stripped eggs from 12 females and one of them was

Danubian wild. In the case of males we got sperm from 12 individuals and one of them was Danubian wild (**Table 2**). The motility of the sperm was checked and only the motile sperm was mixed prior to fertilization. In total 8,004 g of dry eggs were obtained. After fertilization the average fertilization rate was 48.4%.

Table 2. General description and notes for sterlet reproduction in 2020

Sex	Species	PIT tag ID	02/04/2020	06/04/2020	Notes
			Weight (kg)	Reproduction	
♀	**Danube wild sterlet**	0415D4C642	3,65	×	no eggs
	Danube wild sterlet	0415D4C9EB	3,60	✓	569 g eggs
	Sterlet broodstock	0415D4FFB0	5,00	✓	1034 g eggs
	Sterlet broodstock	0415D528D2	4,20	✓	784 g eggs
	Sterlet broodstock	0415D52753	3,95	✓	970 g eggs
	Sterlet broodstock	0415D516FB	3,75	✓	755 g eggs
	Sterlet broodstock	0415D51646	3,75	✓	1060 g eggs
	Sterlet broodstock	0415D52362	3,35	✓	370 g eggs
	Sterlet broodstock	0415D51ADD	3,15	×	no eggs
	Sterlet broodstock	0415D50190	3,15	✓	736 g eggs
	Sterlet broodstock	0415D5237C	3,10	✓	486 g eggs
	Sterlet broodstock	0415D51143	3,10	✓	614 g eggs
	Sterlet broodstock	0415D4AD5C	3,10	✓	264 g eggs
	Sterlet broodstock	0415D5128E	2,75	×	no eggs
	Sterlet broodstock	0415D5208B	2,55	×	no eggs
	Sterlet broodstock	0415D4B2A1	2,50	×	no eggs
♂	**Danube wild sterlet**	0415D4BC62	1,90	×	no sperm
	Danube wild sterlet	0415D4B72E	1,30	×	no sperm
	Danube wild sterlet	0415D4BB98	1,25	✓	
	Danube wild sterlet	0415D4C4AA	1,20	×	no sperm
	Danube wild sterlet	0415D4B615	1,15	×	no sperm
	Danube wild sterlet	0415D4B0F4	1,15	×	no sperm
	Danube wild sterlet	0415D50FB1	1,15	×	no sperm
	Danube wild sterlet	0415D51F79	0,90	×	no sperm
	Sterlet broodstock	0415D4F7E1	5,60	✓	
	Sterlet broodstock	0415D52778	4,00	✓	milted two times
	Sterlet broodstock	0415D5119C	3,35	×	no sperm
	Sterlet broodstock	0415D502CF	2,95	✓	
	Sterlet broodstock	0415D4FE29	2,90	✓	
	Sterlet broodstock	4534647709	2,70	✓	
	Sterlet broodstock	0415D515A8	2,70	✓	
	Sterlet broodstock	0415D49E39	2,70	✓	
	Sterlet broodstock	0415D4DF51	2,65	✓	
	Sterlet broodstock	0415D500ED	2,40	✓	
	Sterlet broodstock	0415D4B063	2,10	✓	
	Sterlet broodstock	0415D4B118	1,80	✓	

3.4. Juvenile reinforcements

Resulting from the two sterlet reproductions conducted in MEASURES at NAIK-HAKI, the broodstock of total 146 individuals was supplemented with juveniles. From 2019, 1,700 juveniles were reared for more than a year, of which 1,500 were used for the sterlet restocking event in 2020 (Baja, Hungary). The remaining juveniles (192 individuals, considering survival) achieved 0.92 kg average body weight until 2020. The reproduction event from 2020 provided an additional 700 juveniles, which have reached a 0.15 kg average body weight until 2020.

The cooperation of the MEASURES project and the LIFE-Sterlet project provided the background for an additional transfer of Danubian sterlet fry and juveniles stemming from wild parents in Austria from BOKU to NAIK-HAKI in 2020. Juveniles hatched in 2018 (2 individuals, 0.4 kg average weight), in 2019 (25 individuals, 0.16 kg average weight) and fry from 2020 (100 pcs, 0.05 kg average weight) were delivered in the summer 2020. Since then, survival was75%, with 2 individuals from the 2018 batch, 24 individuals from the 2019 batch and 77 individuals of the 2020 batch having survived.

3.5. Genetic profiling of sterlet broodstock

For strengthening gene conservation and restocking events, it is important to evaluate the genetic background of the caught wild broodstock. For the analysis to detect variability and genotype microsatellite markers are used. This procedure helps to determine allele-richness and the genetic similarity of individuals from the Danube and its most important tributary, river Tisza.

For this purpose, we assessed the genotype of 144 individuals with 12 microsatellite (DNA) markers from a wild population. The collected fish originated from river Tisza, Kisköre and Mindszent (22 pcs together) and from three sections of river Danube (Leányfalu (D1) – 47 pcs, Ercsi (D2) – 30 pcs, Baja (D3) – 30 pcs and 15 fish originated from the existing *ex situ* stock, this fish was propagated in 2019 and 2020.). The fish were marked with PIT tags, to collect genetic data on the individual level. For the analysis we took samples from the tissue on the caudal fin. DNA isolation was conducted using the E.N.Z.A. kit tissue DNA (Omega Bio-tek Inc.) method (following the instructions set by the producer) and 20-30 mg tissue samples. The quantity and quality of the extracted DNA were assessed using NanoDrop 2000c spectrophotometer (Thermo Scientific, USA). We used 100 ng DNA per sample for the PCR reaction and 12 microsatellite markers for the genetic analysis. During the setting of PCR reaction, we relied on the instructions set by the producer (Multiplex PCR Plus Kit, Qiagen) and optimized the specific settings. The size and quality of the amplified products were assessed on 1.5% agarose and detected the fragments using ABI 3130 sequencing (Applied biosystems, USA). The allele data produced from the electropherograms were identified by the Gene Mapper (Applied Biosystem) software. The specific allele data from the 144 analyzed individuals are presented in **Annex/Table A1**. The specific markers and allele numbers in the table represent the results of the genotyping.

The standard population genetic calculations were delivered by the GenALEx 6.5 (Peakall, Smouse 2012) statistical software package. This was followed by the identification of the genetic distance between populations. Additionally, the assessment of heterzygosity-ratio allowed us to make conclusions regarding the actual genetic structure of the populations. As a result of the genotyping, we detected 118 alleles on the 12 loci. The number of alleles per loci are presented in **Table 3**.

Table 3 The number of alleles for each locus

Locus	Allele number
AN_20	15
AfuG_41	24
ARU_26	8
Spl_163	16
AFUG_51	6
ARU_12	3
Aru 13	21
LS_68	17
Aru_19	2
AOX D_161	12
Aru-50	7
Aru_18	4
Total	135

We analyzed the genetic variability and allelic pattern of populations by loci (**Table 4.**) and calculated averages for populations (**Table 5.**). Different parameters were included in the analysis: number of differentiable genotypes, the relationship between expected and experienced heterozygosity, fixation index. The allele numbers for loci varied between 2 and 24 (11.25 in average).

Table 4 Characteristics of the genetic variability and allelic pattern: number of differentiable genotypes (N), number of alleles (Na), experienced (Ho) and expected (He) heterozygosity, fixation index (F).

Population		AN_20	AfuG_41	ARU_26	Spl_163	AFUG_51	ARU_12	Aru 13	LS_68	Aru_19	AOX D_161	Aru-50	Aru_18
Bajai_D3	N	30	30	30	22	30	30	30	26	30	30	30	30
	Na	9	12	6	9	1	2	7	7	2	5	3	2
	Ho	0,73	0,57	0,47	0,64	0,00	0,97	0,63	0,35	0,37	0,60	0,03	0,23
	He	0,81	0,89	0,66	0,87	0,00	0,50	0,67	0,82	0,34	0,58	0,49	0,21
	F	0,10	0,36	0,29	0,27	0,00	-0,94	0,06	0,58	-0,08	-0,04	0,93	-0,13
Leányfalu_D1	N	43	44	47	45	46	45	46	40	47	47	47	46
	Na	10	17	3	14	5	2	15	16	2	6	6	4
	Ho	0,84	0,82	0,15	0,76	0,17	0,76	0,76	0,43	0,06	0,64	0,89	0,24
	He	0,83	0,91	0,46	0,82	0,27	0,48	0,88	0,92	0,10	0,57	0,63	0,22
	F	-0,01	0,10	0,68	0,08	0,36	-0,57	0,13	0,54	0,37	-0,12	-0,41	-0,11
Ercsi_D2	N	28	28	30	27	30	30	29	29	30	30	30	29
	Na	9	15	3	11	3	2	7	8	2	7	3	2
	Ho	0,64	0,96	0,57	0,22	0,37	0,90	0,55	0,38	0,37	0,80	0,07	0,10
	He	0,79	0,85	0,42	0,82	0,31	0,50	0,65	0,63	0,47	0,78	0,13	0,10
	F	0,19	-0,14	-0,37	0,73	-0,20	-0,80	0,15	0,40	0,22	-0,03	0,47	-0,05
Tiszai	N	20	20	21	21	21	21	21	21	22	22	22	22
	Na	7	9	2	9	4	3	15	12	2	7	3	2
	Ho	1,00	0,80	0,10	0,71	0,24	0,90	0,95	0,62	0,09	0,82	0,86	0,09
	He	0,79	0,84	0,09	0,68	0,22	0,52	0,87	0,89	0,09	0,63	0,54	0,09
	F	-0,27	0,04	-0,05	-0,05	-0,09	-0,75	-0,10	0,30	-0,05	-0,30	-0,60	-0,05
Genbank	N	13	13	14	14	15	14	15	15	15	15	14	15
	Na	10	11	2	6	2	3	8	12	2	7	3	3
	Ho	0,92	0,92	0,36	0,64	0,00	0,86	0,67	0,80	0,27	0,73	0,71	0,27
	He	0,85	0,86	0,38	0,77	0,12	0,56	0,75	0,88	0,23	0,67	0,54	0,24
	F	-0,09	-0,07	0,05	0,16	1,00	-0,53	0,11	0,09	-0,15	-0,10	-0,32	-0,12

Most alleles were detected in the group from Leányfalu, which was followed by populations from Tisza, Ercsi, Genebank and Baja.

Table 5 Genetic variability and averages of the allelic pattern

Population	Bajai_D3	Leányfalu_D1	Ercsi_D2	Tiszai	Genbank
Na	5,42	**8,33**	6	6,25	5,75
Na Freq. >= 5%	4,08	5	3,42	3,25	4
I	1,19	1,39	1,12	1,15	1,22
No. Private Alleles	0,5	**0,92**	0,67	0,42	**0,33**
No. LComm Alleles (<=50%)	0,42	1,17	0,5	0,75	**0,58**
He	0,57	0,59	0,54	0,52	0,57
Ho	0,47	0,54	0,49	0,6	0,6
F	0,13	0,09	0,05	-0,16	0

Values of heterozygosity were used to calculate the F ratio (fixation index), which provides information about the differentiation process of populations. Assuming absolute fixation, that is, the presence of only homozygotes, this value is 1. The average values of fixation index per populations were always close to zero and in the population from Tisza this value was even negative. These results indicate the dominance of heterozygotes in the populations. Among the populations analyzed, the genetic variability between individuals is relatively high and there are no signs for inbreeding. The values from the expected and experienced heterozygosity and their connection also provide important insight regarding the populations. The average expected heterozygosity (He) for the four populations were between 0.52 and 0.57, while the experienced (Ho) values were between 0.47 and 0.61. Comparing population-wise, these values stand close to each other. In the population from Tisza, the averaged experienced heterozygosity exceeded the expected value, which also supports an excess of heterozygotes.

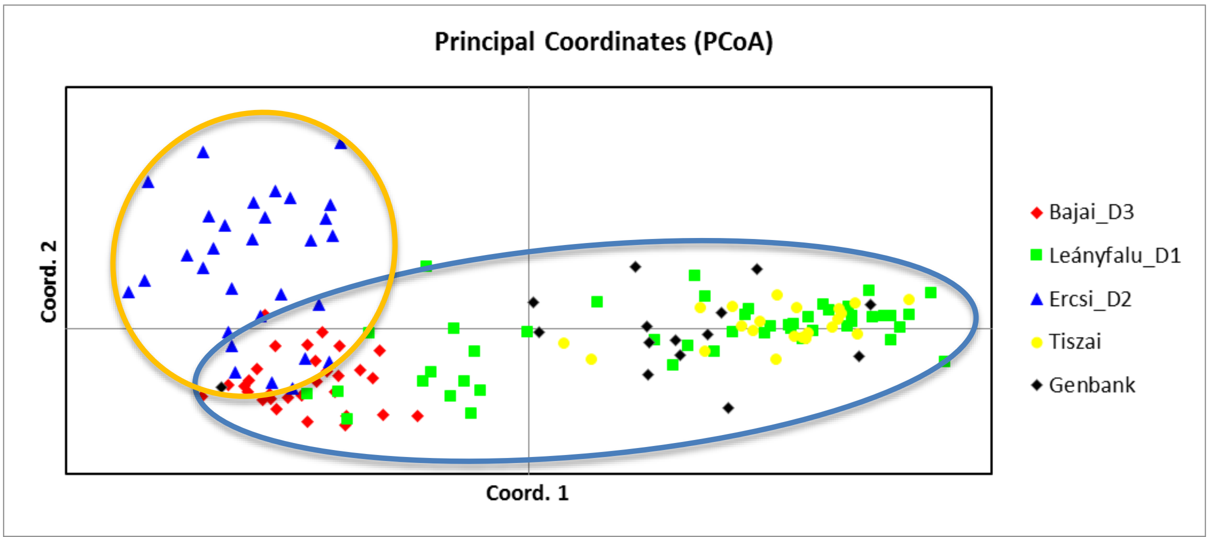

Figure 1. Results of the PCoA test of GenALEx

The genetic differences were calculated using the GENALEX software and we graphically compared the genetic linkages and differences between populations. The results of the PCoA (Principle Coordinate Analysis) analysis are presented in **Figure 1**. The applied procedure assigns characteristic genetic profiles for the groups and then enlists the specific individuals into the most likely groups, based on their calculated genetic value. This can be seen in the figure, where the presentation of individual values helps to explore the genetic relations between the specific groups. The genetic background of the analyzed individuals from the five populations with the applied markers separates two main groups (circled in the PCoA graph). The Leányfalu, Baja, Tisza and Genebank stocks belong to one of them (blue circle) and the other group contains the Ercsi group (orange cicle). The Leányfalu, Baja, Tisza and Genebank populations overlap in population genetic point of view. The genetic pattern of Ercsi stock might refer to the result of previous restocking activities from captured stocks.

4. Russian sturgeon gene stocks

Russian sturgeons are virtually extinct in the Middle-Danube region and NAIK-HAKI has only two Danubian (17.0 kg average weight) and 14 Caspian Russian sturgeons (19.2 kg average weight) as broodstock. The NAIK HAKI additionally purchased 100 pcs Russian sturgeon juveniles with Danube origin from Romania (from 2014 reproduction), since then 95 juveniles survived (1.96 kg average weight) as a potential new generation for broodstock enhancement.

Unfortunately, Russian sturgeon broodstock was not available for the MEASURES project to purchase or obtain from the natural environment. However, the project partners managed to purchase 3,000 fertilized Russian sturgeon eggs with the contribution of five brooders of Danube origin in Romania. The purchase was conducted by the Danube Delta National Institute for Research and Development (DDNI) in early 2020, and the eggs were transported by the Institute of Biodiversity and Ecosystem Research - Bulgarian Academy of Sciences (IBER-BAS) to the University of Natural Resources and Life Sciences, Vienna (BOKU) for hatching. The eggs were incubated and the juveniles reared in Danube water in close cooperation with the LIFE-Sterlet project (LIFE14/NAT/AT/000057). Roughly 1,800 larvae started to feed after pre-larval development. On agreement between project partners, 500 of them were transported to NAIK-HAKI in July for further gene bank enhancement and rearing. Of these 471 survived until September, 2020 and have 0.11 kg average weight. More than 500 stayed in Austria to split the risk and as a basis for further conservation projects. They have an average weight of 0.23 kg as of the end of September. Additionally a little over 300 specimens with around 15 cm TL (total length) have been stocked in the Nationalpark Donauauen in July.

5. Conclusions

Fishing of sterlet from natural waters is experienced more difficult than before and the acquisition of Russian sturgeon individuals is even more troublesome, the MEASURES project successfully increased *ex situ* stocks of both of these species. The new sterlet brooders partially could support the reproduction event in NAIK-HAKI, 2020. Although Russian sturgeon broodstock was not available for purchase, 3,000 fertilized eggs were used by the project partners for gene bank enhancement at BOKU and at NAIK-HAKI as well. The fail-safe recirculation systems provide high protection for fry and juveniles, and the PIT tagging helps to track older individuals to monitor their development closely. The transportation of endangered fish species in MEASURES helped to share the needed infrastructural capacity for mass rearing and also supported the transnational network of multiple, *ex situ* gene banks.

6. References

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7. Annex

Table A1 Allele data from the 144 analyzed individuals

Id	Population	AN_20		AfuG_41		ARU_26		Spl_163		AFUG_51		ARU_12		Aru 13		LS_68		Aru_19		AOX D_161		Aru-50		Aru_18	
B_01	Bajai_D3	176	176	256	256	178	178	214	230	253	253	187	191	123	141	217	217	206	206	126	130	128	138	160	160
B_02	Bajai_D3	172	186	248	248	176	178	226	226	253	253	191	191	123	138	213	213	206	206	126	126	138	138	160	160
B_03	Bajai_D3	164	186	248	260	176	178	222	222	253	253	187	191	123	147	189	189	206	208	126	134	138	138	160	160
B_04	Bajai_D3	172	172	256	256	178	178	226	230	253	253	187	191	123	123	217	217	206	206	126	130	138	138	160	160
B_05	Bajai_D3	186	186	246	250	178	178	222	222	253	253	187	191	135	138	205	205	206	208	154	154	138	138	160	160
B_06	Bajai_D3	180	180	256	260	178	178	214	230	253	253	187	191	123	123	217	229	206	206	126	154	138	138	160	160
B_07	Bajai_D3	172	180	256	260	178	178	222	230	253	253	187	191	123	123	217	217	206	206	126	126	138	138	160	160
B_08	Bajai_D3	164	176	244	244	178	178	226	226	253	253	187	191	147	147	233	233	206	208	126	126	142	142	160	160
B_09	Bajai_D3	176	190	258	258	178	178	226	230	253	253	187	191	141	141	213	217	206	206	126	126	138	138	160	162
B_10	Bajai_D3	176	188	236	236	162	162	0	0	253	253	187	191	123	138	205	205	206	206	126	134	142	142	160	160
B_11	Bajai_D3	176	190	254	258	162	174	0	0	253	253	187	191	123	141	213	229	206	206	126	134	138	138	160	160
B_12	Bajai_D3	176	190	234	254	176	176	0	0	253	253	187	191	123	123	229	229	206	206	126	126	138	138	160	160
B_13	Bajai_D3	176	186	234	256	156	156	0	0	253	253	187	191	123	123	213	229	206	206	126	126	138	138	160	162
B_14	Bajai_D3	172	186	246	246	156	156	0	0	253	253	187	191	123	138	0	0	206	208	126	154	138	138	160	160
B_15	Bajai_D3	176	188	254	254	156	156	0	0	253	253	187	191	123	141	213	229	206	206	126	162	138	138	160	160
B_16	Bajai_D3	164	180	254	258	156	156	0	0	253	253	187	191	138	147	245	245	206	208	126	126	142	142	160	162
B_17	Bajai_D3	188	192	246	254	156	156	0	0	253	253	187	191	123	123	0	0	206	208	126	126	142	142	160	162
B_18	Bajai_D3	172	180	230	230	176	178	224	224	253	253	187	191	141	147	245	245	206	208	126	126	138	138	160	160
B_19	Bajai_D3	176	190	234	258	168	178	210	228	253	253	187	191	123	123	229	229	206	206	126	126	138	138	160	162
B_20	Bajai_D3	176	190	234	246	168	178	210	224	253	253	187	191	108	144	205	205	206	206	126	130	138	138	160	160
B_21	Bajai_D3	176	190	234	258	168	178	210	228	253	253	187	191	123	138	213	217	206	206	126	134	138	138	160	160
B_22	Bajai_D3	176	186	246	258	176	178	210	218	253	253	187	191	123	144	217	217	206	208	126	154	142	142	160	162
B_23	Bajai_D3	172	176	242	254	176	178	224	224	253	253	187	191	123	138	245	245	206	208	126	162	142	142	160	160
B_24	Bajai_D3	176	176	242	254	176	178	228	228	253	253	187	191	123	138	0	0	206	206	126	154	142	142	160	160
B_25	Bajai_D3	176	188	234	258	178	178	224	228	253	253	187	191	138	141	217	217	206	206	126	134	138	138	160	160
B_26	Bajai_D3	186	186	234	242	178	178	210	228	253	253	187	191	123	123	205	213	206	208	130	154	142	142	160	162
B_27	Bajai_D3	172	186	256	256	176	178	214	226	253	253	187	191	123	147	205	213	206	206	126	130	142	142	160	160
B_28	Bajai_D3	164	164	248	248	176	178	210	224	253	253	187	191	123	138	213	213	206	206	126	134	138	138	160	160
B_29	Bajai_D3	176	176	248	248	176	178	238	238	253	253	187	191	123	123	213	245	206	208	126	134	142	142	160	160
B_30	Bajai_D3	176	194	246	246	176	178	210	218	253	253	187	191	135	138	0	0	208	208	154	154	142	142	160	160
D_01	Leányfalu_	180	184	250	254	180	180	208	220	253	260	187	187	108	135	201	201	206	206	122	122	128	138	160	160
D_02	Leányfalu_	176	180	250	254	180	180	208	228	253	253	187	191	123	138	189	189	206	206	122	130	128	138	160	160
D_03	Leányfalu_	176	188	246	264	180	180	208	208	253	253	0	0	138	138	0	0	206	206	122	126	128	138	160	160
D_04	Leányfalu_	176	192	242	254	180	180	208	224	253	253	187	191	135	138	193	193	206	206	122	122	128	138	160	160
D_05	Leányfalu_	172	172	250	266	180	180	0	0	253	253	187	191	126	126	237	237	206	206	122	130	128	138	160	160
D_06	Leányfalu_	176	180	234	246	180	180	226	230	247	253	187	191	138	141	185	185	206	206	126	126	128	138	160	160
D_07	Leányfalu_	172	176	254	262	178	178	222	222	253	253	187	191	90	123	177	185	206	206	122	130	128	138	160	160
D_08	Leányfalu_	172	180	232	250	180	180	222	222	253	253	187	191	90	105	217	217	206	206	122	126	138	138	160	160
D_09	Leányfalu_	172	188	242	242	180	180	208	224	253	253	187	191	126	138	0	0	206	206	122	138	128	138	160	160
D_10	Leányfalu_	172	180	248	248	178	180	208	224	253	253	187	191	123	135	185	193	206	206	122	126	128	138	160	160
D_11	Leányfalu_	172	180	234	250	180	180	218	222	253	253	187	191	138	138	193	193	206	206	122	122	128	128	160	160
D_12	Leányfalu_	0	0	0	0	180	180	208	224	253	253	187	187	105	135	0	0	206	206	122	122	138	138	0	0
D_13	Leányfalu_	176	176	250	258	180	180	208	214	253	260	187	191	135	135	217	221	206	206	122	126	128	138	160	160
D_14	Leányfalu_	180	192	238	242	180	180	208	214	253	260	187	191	108	126	201	229	206	206	122	138	142	142	160	160
D_15	Leányfalu_	176	192	250	258	178	180	208	212	253	253	187	191	99	133	197	209	206	208	122	126	128	138	160	160
D_16	Leányfalu_	176	188	250	262	180	180	208	212	253	253	187	191	99	102	233	233	206	206	122	122	128	138	160	160
D_17	Leányfalu_	172	176	242	258	180	180	208	216	253	253	187	191	123	123	189	205	206	208	122	122	128	138	160	160
D_18	Leányfalu_	172	184	250	262	180	180	208	228	253	253	187	191	126	144	189	201	206	206	122	150	116	122	160	160
D_19	Leányfalu_	0	0	0	0	180	180	208	208	253	253	187	187	105	124	209	217	206	206	122	126	128	138	160	160
D_20	Leányfalu_	180	192	250	262	180	180	208	224	253	253	187	191	108	135	209	209	206	206	126	138	116	128	160	160
D_21	Leányfalu_	172	172	242	246	180	180	208	212	253	253	187	191	126	135	181	181	206	206	122	126	128	138	160	160
D_22	Leányfalu_	0	0	0	0	180	180	216	224	253	253	191	191	108	108	0	0	206	206	122	122	128	138	160	164
D_23	Leányfalu_	176	180	250	266	180	180	224	224	253	253	187	191	0	0	0	0	206	206	122	130	128	138	160	160
D_24	Leányfalu_	176	180	234	258	180	180	208	214	253	253	187	191	123	135	189	189	206	206	122	122	122	128	160	160
D_25	Leányfalu_	184	192	234	248	178	178	208	220	253	253	187	191	132	138	197	205	206	206	122	150	128	138	160	160
D_26	Leányfalu_	172	176	246	246	180	180	208	214	249	253	187	191	135	135	225	225	206	206	126	138	128	138	160	160
D_27	Leányfalu_	176	180	238	254	180	180	206	208	253	253	187	191	123	126	209	209	206	208	122	126	128	138	160	160
D_28	Leányfalu_	176	184	234	254	180	180	208	220	253	253	187	191	123	144	237	237	206	206	122	126	128	138	160	160
D_29	Leányfalu_	0	0	248	248	180	180	0	0	0	0	0	0	105	105	0	0	206	206	122	150	138	138	160	160
D_30	Leányfalu_	172	180	208	208	180	180	208	224	253	253	187	187	124	141	193	205	206	206	122	166	128	138	160	160
D_31	Leányfalu_	176	176	218	232	180	180	214	226	253	253	187	191	90	132	181	181	206	206	126	150	128	138	160	160
D_32	Leányfalu_	180	188	234	254	178	178	208	220	253	263	187	191	123	126	197	209	206	206	122	126	128	138	160	160
D_33	Leányfalu_	172	192	232	238	180	180	212	224	253	253	187	191	108	126	201	205	206	206	122	122	128	138	160	160
D_34	Leányfalu_	176	176	258	262	178	178	208	208	253	253	187	191	99	102	189	197	208	208	122	126	128	138	160	160
D_35	Leányfalu_	172	180	246	266	180	180	208	208	249	253	187	191	99	102	201	201	206	206	122	122	128	138	160	160
D_36	Leányfalu_	176	184	254	258	180	180	208	224	253	253	187	191	99	108	209	209	206	206	122	122	128	138	160	160
D_37	Leányfalu_	170	180	244	254	180	180	208	224	253	253	187	191	123	138	213	225	206	206	122	126	128	138	160	160
D_38	Leányfalu_	170	180	238	266	178	178	210	222	253	253	187	187	123	123	209	209	206	206	122	126	138	124	160	162
D_39	Leányfalu_	176	188	254	258	176	178	214	234	253	253	187	191	108	138	0	0	206	206	122	126	138	142	160	162
D_40	Leányfalu_	176	192	238	258	176	178	226	226	253	253	187	191	123	123	213	229	206	206	122	126	138	142	160	162
D_41	Leányfalu_	162	162	254	254	176	178	222	222	253	253	187	187	135	147	197	225	206	206	122	130	138	142	160	162

Id	Population	AN_20		AfuG_41		ARU_26		Spl_163		AFUG_51		ARU_12		Aru 13		LS_68		Aru_19		AOX D_161		Aru-50		Aru_18	
D_43	Leányfalu_	176	184	236	260	176	178	222	226	253	253	187	187	123	138	213	213	206	206	122	122	138	142	160	162
D_44	Leányfalu_	170	180	242	250	178	178	212	224	249	249	187	187	123	135	185	185	206	206	126	126	138	142	160	162
D_45	Leányfalu_	180	186	234	246	178	178	220	224	249	249	187	191	135	138	225	225	206	206	122	130	138	142	160	162
D_46	Leányfalu_	182	186	266	266	176	178	216	220	249	249	187	191	123	123	221	229	206	206	122	122	138	142	160	162
D_47	Leányfalu_	176	184	248	248	178	178	218	218	253	253	187	187	138	138	217	217	206	206	122	122	138	142	158	160
E_01	Ercsi_D2	186	194	254	258	178	178	226	226	253	253	187	191	123	138	209	209	206	206	126	134	138	138	160	162
E_02	Ercsi_D2	172	190	244	256	176	178	222	226	253	253	187	191	123	123	213	213	206	208	126	130	128	138	0	0
E_03	Ercsi_D2	172	186	260	272	178	178	226	230	253	253	187	187	123	138	209	209	206	206	126	134	138	138	160	162
E_04	Ercsi_D2	0	0	0	0	178	188	232	232	247	253	191	191	126	126	225	225	206	206	126	142	138	138	160	160
E_05	Ercsi_D2	176	194	254	258	178	178	208	208	253	253	187	191	126	126	225	225	208	208	138	138	138	138	160	160
E_06	Ercsi_D2	194	194	232	250	178	188	208	208	253	253	187	191	123	123	225	225	208	208	142	142	128	138	160	160
E_07	Ercsi_D2	176	194	232	254	178	188	208	208	253	253	187	191	123	126	225	225	206	208	126	130	138	138	160	160
E_08	Ercsi_D2	176	194	232	252	178	188	216	216	253	253	187	191	123	126	221	225	206	208	126	138	138	138	160	160
E_09	Ercsi_D2	0	0	0	0	178	188	208	208	253	253	187	191	126	126	221	225	208	208	138	138	138	138	160	160
E_10	Ercsi_D2	184	194	250	258	178	188	0	0	253	253	187	191	126	126	201	225	206	206	138	138	138	138	160	160
E_11	Ercsi_D2	176	194	232	254	178	178	0	0	247	253	187	191	123	123	221	225	206	208	126	138	138	138	160	160
E_12	Ercsi_D2	184	190	232	264	178	178	214	214	253	253	187	191	123	126	213	217	206	206	126	130	140	140	160	160
E_13	Ercsi_D2	194	194	248	258	178	178	226	226	253	260	187	191	123	123	189	189	206	206	126	130	138	138	160	160
E_14	Ercsi_D2	176	194	232	259	178	188	208	208	247	253	187	191	123	123	225	225	206	208	138	142	138	138	160	160
E_15	Ercsi_D2	180	184	248	258	178	178	222	238	247	253	187	191	135	141	205	209	206	208	126	130	138	138	160	160
E_16	Ercsi_D2	184	184	232	232	178	178	208	208	253	253	187	191	126	138	225	225	206	208	138	154	138	138	160	160
E_17	Ercsi_D2	194	194	254	258	178	188	216	216	247	253	187	191	123	123	221	225	206	208	126	126	138	138	160	160
E_18	Ercsi_D2	182	194	246	258	178	188	208	208	247	253	187	191	126	126	225	225	206	206	126	142	138	138	160	160
E_19	Ercsi_D2	194	194	232	260	178	188	234	234	247	253	187	191	123	126	225	225	208	208	138	158	138	138	160	160
E_20	Ercsi_D2	176	194	250	258	178	188	216	216	247	253	187	191	126	126	201	205	208	208	126	138	138	138	160	160
E_21	Ercsi_D2	186	186	238	258	178	178	208	216	253	253	187	191	123	133	225	225	206	206	126	154	138	138	160	160
E_22	Ercsi_D2	186	186	232	250	178	188	0	0	253	253	187	191	126	126	209	225	206	208	130	138	138	138	160	160
E_23	Ercsi_D2	176	194	250	258	178	188	226	226	253	253	187	191	123	126	201	225	208	208	126	130	138	138	160	160
E_24	Ercsi_D2	176	194	254	258	178	188	224	224	253	253	187	191	123	126	201	225	206	206	126	154	138	138	160	160
E_25	Ercsi_D2	168	172	250	254	178	178	216	218	253	253	187	191	136	138	205	205	206	206	126	126	138	138	160	160
E_26	Ercsi_D2	182	182	232	254	178	188	224	224	253	253	191	191	0	0	0	0	206	208	126	130	138	138	160	160
E_27	Ercsi_D2	186	194	238	254	178	178	216	224	247	253	187	191	123	126	225	225	206	206	134	154	138	138	160	160
E_28	Ercsi_D2	176	176	246	258	178	188	232	232	247	253	187	191	123	126	225	225	206	208	138	154	138	138	160	160
E_29	Ercsi_D2	176	186	219	232	178	178	208	208	253	253	187	191	126	138	225	225	206	206	138	154	138	138	160	160
E_30	Ercsi_D2	172	172	254	258	178	178	224	224	253	253	187	191	123	136	225	225	206	206	126	134	138	138	160	162
T-01	Tiszai	176	184	254	254	180	180	208	220	253	253	187	191	138	150	193	201	206	206	122	130	128	138	160	164
T_02	Tiszai	176	184	254	258	178	180	208	208	253	253	187	191	123	138	213	221	206	208	122	126	142	142	160	164
T_03	Tiszai	176	180	234	234	180	180	208	226	253	253	187	191	123	135	221	225	206	206	126	138	128	138	160	160
T_04	Tiszai	172	188	250	254	178	180	208	226	253	259	187	191	136	138	189	189	206	206	122	122	128	138	160	160
T_05	Tiszai	176	192	250	262	180	180	208	208	0	0	0	0	108	135	213	233	206	206	122	126	138	138	160	160
T_06	Tiszai	176	188	250	258	180	180	208	208	253	253	187	187	126	135	185	197	206	206	122	126	128	138	160	160
T_07	Tiszai	172	192	254	258	180	180	214	232	253	253	187	191	123	153	185	193	206	206	122	162	128	138	160	160
T_08	Tiszai	180	184	238	246	180	180	208	226	253	253	187	191	123	138	209	233	206	206	122	126	128	138	160	160
T_09	Tiszai	0	0	0	0	180	180	208	214	253	253	187	191	123	129	189	189	206	206	122	126	128	138	160	160
T_10	Tiszai	180	192	238	254	180	180	220	220	253	253	187	191	123	126	205	213	206	206	126	150	128	138	160	160
T_11	Tiszai	0	0	0	0	180	180	206	208	253	253	187	191	108	144	205	213	206	206	122	122	128	138	160	160
T_12	Tiszai	176	180	246	250	180	180	208	220	249	253	187	191	126	138	189	201	206	206	122	154	128	138	160	160
T_13	Tiszai	180	184	246	258	180	180	208	226	253	260	187	187	123	147	185	201	206	206	126	130	128	138	160	160
T_14	Tiszai	176	188	250	250	180	180	208	226	253	253	187	191	99	135	189	197	206	206	122	126	128	138	160	160
T_15	Tiszai	172	176	234	234	0	0	0	0	253	253	187	191	0	0	0	0	206	206	122	130	128	138	160	160
T_16	Tiszai	172	176	254	258	180	180	208	208	253	253	187	191	108	138	189	189	206	206	122	126	128	138	160	160
T_17	Tiszai	176	180	250	254	180	180	212	212	253	253	187	191	126	138	217	217	206	206	122	126	128	138	160	160
T_18	Tiszai	176	184	254	258	180	180	208	212	253	253	187	191	132	138	213	213	206	206	122	122	128	138	160	160
T_19	Tiszai	162	176	254	262	180	180	208	226	253	253	187	194	105	105	209	225	206	206	122	130	128	138	160	160
T_20	Tiszai	176	180	208	278	180	180	208	224	253	260	187	191	123	123	217	217	206	206	122	122	128	138	160	160
T_21	Tiszai	172	180	238	246	180	180	208	220	253	253	187	191	120	123	193	193	206	206	122	126	138	138	160	160
T_22	Tiszai	176	192	250	258	180	180	208	216	249	253	187	191	129	138	217	217	206	208	122	126	128	138	160	160
G_01	Genbank	176	184	234	242	180	180	222	226	253	253	187	191	108	123	189	189	206	206	122	126	138	138	160	160
G_02	Genbank	172	194	238	246	178	180	208	222	253	253	187	191	123	123	197	201	206	208	122	162	128	138	160	160
G_03	Genbank	0	0	0	0	178	180	208	222	253	253	187	191	126	138	229	205	206	208	122	150	128	138	160	160
G_04	Genbank	164	176	236	244	178	178	222	222	253	253	191	191	123	138	205	205	206	206	126	126	138	138	160	162
G_05	Genbank	186	186	232	242	0	0	0	0	253	253	0	0	138	138	213	225	206	206	122	122	138	138	160	160
G_06	Genbank	172	184	254	270	178	180	208	214	253	253	187	191	123	138	193	201	206	206	122	130	128	138	160	164
G_07	Genbank	0	0	0	0	180	180	208	208	253	253	187	191	123	123	209	217	206	208	122	130	128	138	160	160
G_08	Genbank	188	192	246	254	180	180	214	228	253	253	187	191	123	123	225	237	206	206	122	154	128	138	160	160
G_09	Genbank	186	192	254	270	180	180	226	226	253	253	187	191	108	128	181	189	206	206	122	126	142	142	160	164
G_10	Genbank	176	192	246	246	180	180	208	228	253	253	187	191	123	135	197	205	206	208	122	154	0	0	160	160
G_11	Genbank	180	192	236	254	178	180	208	226	253	253	187	191	138	138	213	225	206	206	122	154	128	138	160	160
G_12	Genbank	172	192	254	258	180	180	226	226	253	253	187	191	102	138	181	189	206	206	122	122	128	138	160	160
G_13	Genbank	180	192	236	258	178	180	206	226	260	260	194	194	138	150	189	189	206	206	122	154	128	138	160	160
G_14	Genbank	174	186	246	254	180	180	226	226	253	253	187	191	123	135	197	205	206	206	126	154	128	138	160	164
G_15	Genbank	176	192	208	242	180	180	208	214	253	253	187	191	108	135	225	229	206	206	122	122	128	138	160	160